BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

space[9] rather than axonal transport (S.B. and A.A., unpublished results).

It has been argued that scrapie pathology is due to intrinsic neurotoxicity of PrP^{Sc} rather than to depletion of PrP^{C} because *Prn-p*$^{o/o}$ mice are normal in their development and behaviour[3,10], deposition of PrP-immunoreactive material colocalizes with typical histopathology[11], and synthetic amyloidogenic PrP fragments as well as liposome-packaged PrP^{27-30} (the protease-resistant core protein of PrP^{Sc}) are neurotoxic *in vitro*[12,13]. However, even in regions adjoining the graft, which contained high levels of PrP^{Sc} and was clearly leaking such material, no scrapie pathology was observed in PrP^{C}-deficient tissue. Perhaps PrP^{Sc} is inherently non-toxic and PrP^{Sc} plaques found in spongiform encephalopathies are an epiphenomenon rather than a cause of neuronal damage. Indeed, the extent of PrP deposition in the brains of humans succumbing to prion diseases with similar clinical presentation is extremely variable[14,15]. Alternatively, PrP^{Sc} is only toxic when it is formed and accumulated within the cell, but not when it is presented from outside. Finally, it may be that PrP^{Sc} is pathogenic when presented from outside, but only to cells expressing PrP^{C}, either because it initiates conversion of PrP^{C} to PrP^{Sc} at the cell surface and/or because it is internalized by way of association with PrP^{C}, which is endocytosed efficiently[16]. Considered together with previous transgenic studies[17,18] that show delayed onset of disease in *Prn-p*$^{o/+}$ mice despite massive accumulation of PrP^{Sc}, and with reports of typical scrapie histopathology in fatal familial insomnia-inoculated mouse brains in which PrP^{Sc} cannot be detected[19], our data imply that it is not deposition of PrP^{Sc} but rather the availability of PrP^{C} for some intracellular process elicited by the infectious agent that is directly linked to spongiosis, gliosis and neuronal death. □

Received 15 August; accepted 15 November 1995.

1. Prusiner, S. B. *A. Rev. Microbiol.* **48**, 655–686 (1994).
2. Weissmann, C. *Trends Cell Biol.* **4**, 10–14 (1994).
3. Büeler, H. R. *et al. Nature* **356**, 577–582 (1992).
4. Büeler, H. R. *et al. Cell* **73**, 1339–1347 (1993).
5. Sailer, A., Büeler, H., Fischer, M., Aguzzi, A. & Weissmann, C. *Cell* **77**, 967–968 (1994).
6. Fischer, M. *et al. EMBO J.* (in the press).
7. Taraboulos, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **89**, 7620–7624 (1992).
8. Isenmann, S., Brandner, S., Sure, U. & Aguzzi, A. *Neuropathol. appl. Neurobiol.* (in the press).
9. Jeffrey, M. *et al. Neurosci. Lett.* **174**, 39–42 (1994).
10. Manson, J. C. *et al. Molec. Neurobiol.* **8**, 121–127 (1994).
11. Jeffrey, M *et al. Brain Res.* **656**, 329–343 (1994).
12. Forloni, G. *et al. Nature* **362**, 543–546 (1993).
13. Müller, W. E. *et al. Eur. J. Pharmac.* **248**, 261–267 (1993).
14. Collinge, J. *et al. Lancet* **336**, 7–9 (1990).
15. Hayward, P. A., Bell, J. E. & Ironside, J. W. *Neuropath. appl. Neurobiol.* **20**, 375–383 (1994).
16. Shyng, S. L., Huber, M. T. & Harris, D. A. *J. biol. Chem.* **268**, 15922–15928 (1993).
17. Bueler, H. *et al. Molec. Med.* **1**, 19–30 (1994).
18. Manson, J. C., Clarke, A. R., McBride, P. A., McConnell, I. & Hope, J. *Neurodegeneration* **3**, 331–340 (1994).
19. Collinge, J. *et al. Lancet* **346**, 569–570 (1995).
20. Reed, J. & Muench, H. *Am. J. Hyg.* **27**, 493–497 (1938).
21. Prusiner, S. B. *et al. Ann. Neurol.* **13**, 353–358 (1982).
22. Farquhar, C. F., Somerville, R. A. & Ritchie, L. A. *J. virol. Meth.* **24**, 215–221 (1989).
23. Schaeren-Wiemers, N. & Gerfin-Moser, A. *Histochemistry* **100**, 431–440 (1993).

ACKNOWLEDGEMENTS. We thank O. D. Wiestler and P. Gambetti for critical comments and M. König for technical help. This study has been supported by the kanton of Zürich, by grants from the Schweizerischer Nationalfonds (A.A. and C.W.), the European Union (A.A.) the Human Frontier Science Program (C.W.) and by a postdoctoral fellowship of the Ernst Hadorn Foundation to S.B.

Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein

Stefan Brocke*, Koenraad Gijbels*, Mark Allegretta*, Iris Ferber†, Christopher Piercy*, Thomas Blankenstein‡, Roland Martin§, Ursula Utz§, Nathan Karin*, Dennis Mitchell*, Timo Veromaa‖, Ari Waisman¶, Amitabh Gaur†, Paul Conlon†, Nicholas Ling†, Paul J. Fairchild#, David C. Wraith#, Anne O'Garra, C. Garrison Fathman† & Lawrence Steinman*¶††**

Departments of *Neurology and Neurological Sciences, †Medicine and ‖Pathology, Stanford University Medical Center, Stanford, California 94305, USA
‡Max-Delbrueck-Centrum for Molecular Medicine, 13125 Berlin, Germany
§Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, USA
¶Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel
†Neurocrine Biosciences, La Jolla, California 92121 USA
#Department of Pathology, Cambridge University, Cambridge CB2 1QP, UK
**DNAX Research Institute, Palo Alto, California 94305, USA

Following induction of experimental encephalomyelitis with a T-cell clone, L10C1, that is specific for the myelin basic protein epitope p87–99, the inflammatory infiltrate in the central nervous system contains a diverse collection of T cells with heterogeneous receptors. We show here that when clone L10C1 is tolerized *in vivo* with an analogue of p87–99, established paralysis is reversed, inflammatory infiltrates regress, and the heterogeneous T-cell infiltrate disappears from the brain, with only the T-cell clones that incited disease remaining in the original lesions. We found that antibody raised against interleukin-4 reversed the tolerance induced by the altered peptide ligand. Treatment with this altered peptide ligand selectively silences pathogenic T cells and actively signals for the efflux of other T cells recruited to the site of disease as a result of the production of interleukin-4 and the reduction of tumour-necrosis factor-α in the lesion.

The T-cell clone L10C1 recognizes the myelin basic protein (MBP) epitope p87–99 and causes severe experimental encephalomyelitis (EAE) (Table 1). The third complementary-determining regions (CDR3) in the T-cell antigen receptor (TCR) α and β-chains of L10C1 are Vβ6-PRGVGNQ and Vα1-SDTG, respectively. These sequences are homologous to VDJβ or VNJα sequences in human or rat T-cell clones specific for MBP p87–99, and with VDJβ and VNJα sequences found in multiple sclerosis (MS) lesions[1–7]. We tolerized mice paralysed with EAE by targeting the T-cell clones that initiated disease; we then investigated the dynamics of the inflammatory infiltrates within the central nervous system (CNS).

$(PL/JxSJL/J)F_1$ mice received L10C1 and were treated with altered peptide ligand (APL) directly after transfer or after paralysis was established. Administration of the APL p87–99 (96P → A), in which a phenylalanine at residue 96 was replaced by alanine, prevented EAE or reversed paralysis (Table 1). This peptide binds poorly to $I\text{-}A^{s}$ (Table 1) and induces a lower proliferative response in L10C1 than p87–99 (data not shown). In contrast, soluble non-stimulatory peptide analogues of p87–99, lacking residues critical for binding to the TCR or major histocompatibility complex (MHC), for example p87–99 (91K → A), p87–99 (92N → A), or p87–99 (93I → A), had no effect on disease (Table 1). The peptide p87–99 (90F → A) increased MHC binding relative to p87–99 and altered T-cell signalling (Table 1). A T-cell line reactive to p87–99 (90F → A) abrogated EAE induced with spinal cord homogenate (data not shown).

The mechanism causing tolerance was analysed. p87–99 (96P → A) is a partial agonist for L10C1 (Table 1). The therapeutic APL had no influence on the *in vitro* proliferation of L10C1 by p87–99 (data not shown), so MHC competition[8] or TCR antagonism[9] are unlikely to explain its action.

†† To whom correspondence should be addressed.

TABLE 1 Stimulation of MBP p87-99-specific T-cell clone L10C1 by peptides with single amino-acid substitutions

Peptide		Substitution	Relative affinity for I-A^u	Proliferation of L10C1	Treatment of EAE
87-99		VHFFKNIVTPRTP	++	+++	+
87-99	87 A	AHFFKNIVTPRTP	++	++	ND
87-99	88 A	VAFFKNIVTPRTP	++	++	ND
87-99	89 A	VHAFKNIVTPRTP	+	+-	ND
88-99	90 A	VHFAKNIVTPRTP	+++	-	-
87-99	91 A	VHFFANIVTPRTP	ND	-	-
87-99	92 A	VHFFKAIVTPRTP	+	-	-
87-99	93 A	VHFFKNAVTPRTP	+++	-	-
87-99	94 A	VHFFKNIATPRTP	+++	-	ND
87-99	95 A	VHFFKNIVAPRTP	+++	+	ND
87-99	96 A	VHFFKNIVTARTP	+	+	+
87-99	97 A	VHFFKNIVTPATP	ND	+	ND
87-99	98 A	VHFFKNIVTPRAP	ND	+	ND
87-99	99 A	VHFFKNIVTPRTA	ND	-	ND

Human MBP peptide p87-99 and a series of single alanine-substituted analogues were synthesized as described[19]. The relative affinity of peptides was determined using the I-A^u-expressing B-cell lymphoma line LS102.9. The line was maintained in IMDM medium (Gibco) supplemented with 5% FCS (Flow Lab), 2 mM L-glutamine (Gibco), 5×10^{-5} M 2-mercaptoethanol, 100 U ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin (IMDM-5). Cells were collected while subconfluent and distributed at 2×10^5 cells per well of a 96-well microtitre plate. Cells were resuspended in IMDM-5 containing a top concentration of the biotinylated peptide, Bio87-99, of 100 μM. Alanine-substituted analogues of p87-99 were added to 300 μM and cells were incubated at 37 °C for 75 min. Unbiotinylated p87-99 was used as a positive control for competition binding studies; the I-A^u-restricted peptide Ac1-9 (4K—Y) was used as a negative control. LS102.9 cells were then washed three times in PBS supplemented with 1% FCS and 2 mM NaN_3 (washing solution) before being incubated on ice in ExtrAvidin-FITC (Sigma) for 30 min. Cells were washed thoroughly before being fixed in 1% formaldehyde (BDH). Three thousand events from each sample were analysed on a Becton Dickinson FACSort using Lysis II software and mean channel fluorescence was used as a measure of fluorescence intensity. Binding was scored as follows: -, no binding; +, binding with an affinity intermediate between Ac1-9 (4K—Y) and p87-99; ++, binding equivalent to p87-99; +++, binding with an affinity greater than p87-99. T-cell proliferation was assayed as described[20] and the transfer of EAE by T-cell clone L10C1 and treatment with soluble peptide analogues are described in Fig. 1 legend. T-cell responses were graded as: +, proliferation at 40 μM peptide; ++, proliferation at 4 μM peptide; +++, proliferation at 1 μM peptide. Treatment of EAE. + refers to the ability to prevent or reverse ongoing EAE (see legends to Figs 1 and 2) by injection of the APL or native p87-99 intrapentoneally. ND, not determined.

In EAE, inflammatory infiltrates are heterogeneous[10], with a diverse array of TCR *V*-region genes being seen seven days after the transfer of L10C1 (Table 2*a*). When treated with APL after onset of paralysis, the diversity of the TCR transcripts in infiltrates was reduced in the spinal cord, whereas the different TCR transcripts disappeared within 48 h in the brain (Table 2*a*). Furthermore, tumour-necrosis factor (TNF)-α transcripts, which are associated with pathogenicity of T-cell clones[11,12], cannot be detected in brain after treatment with APL (Table 2*b*). When scoring inflammatory infiltrates, there were significant differences between mice treated with p87-99 (96P → A) compared to controls (data not shown).

Direct sequencing of *V*β*6* transcripts from brains of diseased animals after reversal of EAE demonstrated that the TCR VDJβ sequence, Vβ6PRGVGNQ, of residual cells in the lesions was identical to that of the clone initially transferred into the mice. Thus, the disease mediated by the T-cell clone can still be positively identified *in situ* after treatment with APL, whereas other inflammatory T cells had disappeared. Because it was possible directly to sequence Vβ6-Cβ PCR products from the EAE lesion, there must have been little or no diversity in Vβ6 TCR transcripts following APL treatment. The presence of the encephalitogenic clone in the lesion of animals successfully treated with the APL is insufficient to maintain clinical disease. These residual T cells have been silenced. The presence of full-length Vβ6-D-J-Cβ sequences indicates that apoptosis has not

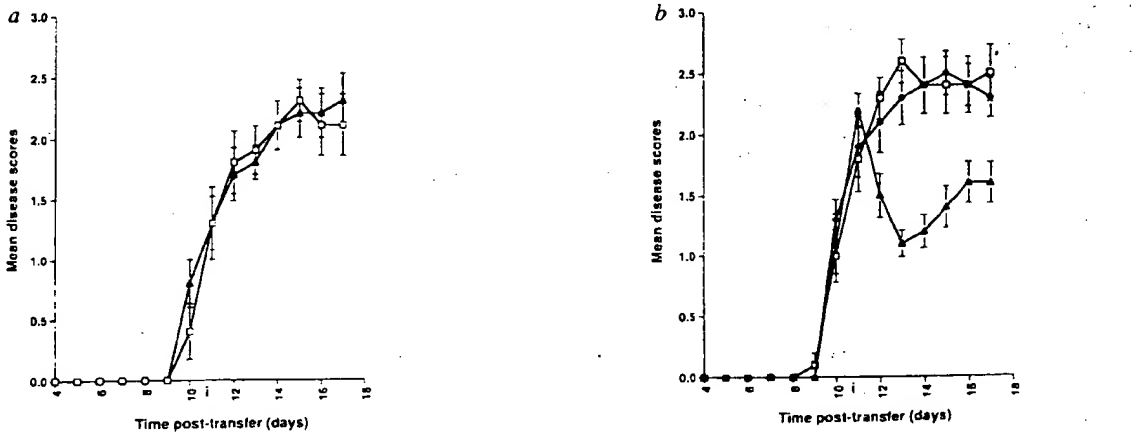

FIG. 1 Reversal of adoptively transferred EAE with MBP analogue p87-99(96P → A). *a*, Soluble MBP analogue p87-99 (96P → A) has no effect on EAE mediated by MBP Ac1-11-specific T-cell line L9. Ten animals per group were treated with PBS alone (squares) or with 0.5 mg soluble p87-99(96P → A) (triangles) in 0.5 ml PBS. *b*, Soluble MBP analogue p87-99(96P → A) reduces severity of EAE mediated by the Ac1-11-specific T-cell line L9 when T-cell clone L10C1 is transferred as well. Ten animals per group were treated with PBS alone (squares), with 0.5 mg soluble p87-99(91K → A) (diamonds) in 0.5 ml PBS, or 0.5 mg soluble p87-99(96P → A) (triangles) in 0.5 ml PBS (↑, time of i.p. injection of peptides). METHODS. EAE was induced by transferring 3×10^6 T cells of the MBP Ac1-11 specific T-cell line L9 (ref. 20). On day 6 after transfer, groups of mice shown in *b* were injected with 5×10^6 T cells of T-cell clone L10C1. Eleven days after the first transfer to induce EAE, mice were injected i.p. with 0.5 mg in 0.5 ml PBS of MBP peptide analogues p87-99(96P → A), p87-99(91K → A) or PBS alone, as indicated. Mice were scored daily according to the following scale: 0, no clinical disease; 1, tail weakness; 2, mono- or paraparesis (incomplete paralysis of one or two hindlimbs); 3, mono- or paraplegia (complete paralysis of one or two hindlimbs); 4, paraplegia with forelimb weakness or paralysis; 5, moribund or dead animals. Data are given as mean disease scores ±s.e.

TABLE 2 TCR V and TNF-α gene expression in the CNS of (PL/JxSJL/J) F_1 mice after treatment with p-87–99 peptide analogues

Treatment/sample	TCR V_β 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
p87–99 (96P→A)																			
Spinal cord 1	+	+	+			+		+		+		+	+			+	+		
Spinal cord 2		+				+			+				+						
Control peptides																			
Spinal cord 3	+	+				+		+	+			+	+		+				
Spinal cord 4	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+		+	
P87–99 (96p→A)																			
Brain 1						+					+								
Brain 2						+													
Control peptides																			
Brain 3	+	+				+	+	+	+						+		+		
Brain 4	+					+		+			+	+		+	+		+		

Treatment/sample	TNF-α	β-Actin
p87–99 (96P→A)		
Spinal cord 1	+	+
Spinal cord 2	+	+
Control peptides		
Spinal cord 3	+	+
Spinal cord 4	+	+
p87–99 (96P→A)		
Brain 1	–	+
Brain 2	–	+
Control peptides		
Brain 3	+	+
Brain 4	+	+

The encephalitogenic T-cell clone L10C1 was activated *in vitro* with MBP p87–99 and antigen-presenting cells and adoptively transferred into mice. All mice developed paralysis on day 7 after transfer, with an EAE score of at least 2. On day 7 and/or 8 and 9, mice were treated intraperitoneally with 0.5 mg of soluble peptide analogues p87–99(96P→A) or soluble control peptides (p87–99(92N→A) or p87–99(93I→A)) as indicated. 48 h after treatment, mice were killed by CO_2 inhalation and then perfused with 30 ml PBS and their brains and spinal cords dissected. Reverse-transcribed mRNA isolated from CNS tissue was analysed by PCR using either TCR Vβ primers specific for 19 different mouse TCR Vβ regions and a single common Cβ primer, or β-actin primers and mouse TNF-α-specific primers. Perfusion of animals, preparation and storage of CNS tissue, RNA and cDNA preparation from tissue samples, PCR amplification of cDNA using β-actin primers and TCR Vβ primers specific for 19 different mouse TCR Vβ regions have all been described[20]. Primers were synthesized by Operon Technologies (Alameda, CA). TCR products amplified by PCR were confirmed by Southern blotting using a Cβ-specific probe. PCR amplification of CNS tissue derived RNA/cDNA using mouse TNF-α-specific primers (TNF-α sense primer: 5'-GGC AGG TCT ACT TTG GAG TCA TTG C-3'; TNF-α antisense primer: 5' ACA TTC GAG GCT CCA GTG AAT TCG G-3) has been described[21].

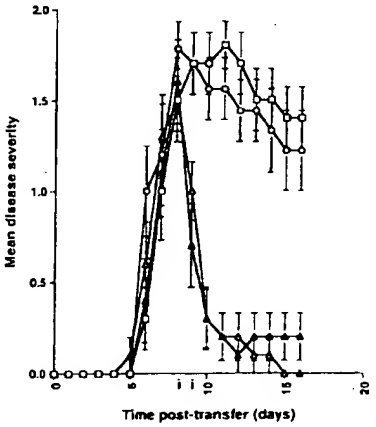

FIG. 2 Reversal of adoptively transferred EAE with MBP analogue p87–99(96P → A) is dependent on IL-4 *in vivo*. Nine to ten animals per group were injected with 10×10^6 T cells of T-cell clone L10C1. On day 8 and 9 after transfer, mice were treated with 0.5 ml PBS (squares) or 0.5 mg soluble p87–99(96P → A) (triangles and circles) in 0.5 ml PBS. Two hours later, PBS (black triangles), 1 mg HPLC-purified mABs 11B11 (rat anti-mouse-IL 4, IgG1, circles) or GL113 (rat-anti-mouse-β-galactosidase, IgG1; open triangles) were injected i.p. as indicated in (i, time of i.p. injection of peptides and antibodies). Mice were scored daily as described in Fig. 1 legend. Data are given as mean disease scores ± s.e.

deleted all T-cell clones responsible for inciting disease. We searched for other mechanisms underlying tolerance.

We asked whether antigen-specific immunotherapy with APL therapy is confined to the specific T cells that recognize APL. If so, the therapeutic potential of this approach would be limited in the case of diverse antigen reactivity[13]. Figure 1 shows that EAE mediated by MBPpAc1-11-specific T cells can be ameliorated by targeting p87–99-specific T cells with p87–99 (96P → A). This protection only occurs if L10C1 T cells, reactive to p87–99, are present in the lesions after co-transfer (Fig. 1*b*), indicating that APL can directly influence T cells with other specificities in an established lesion. The *trans*-acting effect depends on the intralesional presence of a T-cell clone that recognizes the APL.

Reversal of EAE with p87–99 (96P → A) depends on the availability of the cytokine interleukin-(IL)-4. Neutralizing monoclonal antibodies directed against IL-4 block the therapeutic effect of APL (Fig. 2). In parallel studies, EAE induced with proteolipid protein can be reversed at the onset of paralysis with the proteolipid protein PLP p139–151 given intraperitoneally. This effect is abrogated when anti-IL-4 is given together with p139–151 (data not shown).

L10C1 produced both IL-4 and TNF-α. T cells (10^6 per ml medium) from L10C1 were cultured for 36 hours, together with 10×10^6 irradiated syngeneic splenocytes and 10 μM MBP p87–99 or p87–99 (P → A): 18.680 ± 57 pg ml^{-1} or 880 ± 57 pg ml^{-1}, respectively, of TNF-α and 77.5 ± 3.5 pg ml^{-1} or 13.5 ± 2.1 pg ml^{-1}, respectively, of IL-4 were detected. Thus the APL p87–99 (96P → A) shifts the ratio between TNF-α and IL-4 by ~4-fold. IL-4 is a potent inhibitor of TNF-α (ref. 14).

Inflammation is a dynamic process, and T-cell tolerance with APL leads to the regression and dissolution of an inflammatory

infiltrate. This phenomenon is mediated by the downregulation of the production of TNF-α and by the action of IL-4. TNF-α is a mediator of cell recruitment in inflammatory infiltrates[15] and is a critical cytokine in the pathogenesis of EAE, and probably of multiple sclerosis[16]; IL-4 is associated with recovery from disease, and is seen in multiple sclerotic lesions[17,18]. By selectively targeting the initial trigger for inflammation, the secondary infiltrate can be controlled. This may be relevant for highly selective immune therapy of autoimmune disease in the face of determinant spreading[13] and nonspecific amplification of the inflammatory response. □

Received 18 September; accepted 14 December 1995.

1. Oksenberg, J. R. *et al. Nature* **362,** 68–70 (1993).
2. Steinman, L. *Behring Inst. Mitt.* **94,** 148–157 (1994).
3. Martin, R. *et al. J. exp. Med.* **173,** 19–24 (1991).
4. Gold, D. P. *et al. J. Immun.* **148,** 1712–1717 (1992).
5. Vogt, A. B. *et al. J. Immun.* **153,** 1665–1673 (1994).
6. Koop, B. F. *et al. Genomics* **13,** 1209–1230 (1992).
7. Wucherpfennig, K. W. *et al. J. exp. Med.* **179,** 279–290 (1994).
8. Wraith, D. C., McDevitt, H. O., Steinman, L. & Acha-Orbea, H. *Cell* **57,** 709–715 (1989).
9. De Magistris, M. T. *et al. Cell* **68,** 625–634 (1992).
10. Bell, B. B., Lindsey, J., Sobel, R. A., Hodgkinson, S. & Steinman, L. *J. Immun.* **150,** 4085–4092 (1993).
11. Selmaj, K. W., Farooq, M., Norton, W. T., Raine, C. S. & Brosnan, C. F. *J. Immun.* **144,** 129–136 (1990).
12. Powell, M. B. *et al. Int. Immun.* **2,** 539–544 (1990).
13. Lehmann, P. V., Forsthuber, T., Miller, A. & Sercarz, E. E. *Nature* **358,** 155–157 (1992).
14. Fiorentino, D. F., Zlotnik, A., Mosmann, T. R., Howard, M. & O'Garra, A. *J. Immun.* **147,** 3815–3822 (1991).
15. Vassalli, P. *Rev. Immun.* **10,** 411–452 (1992).
16. Raine, C. S. *Nature Med.* **1,** 211–214 (1995).
17. Racke, M. *et al. J. exp. Med.* **180,** 1961–1966 (1994).
18. Cannella, B. & Raine, C. S. *Ann. Neurol.* **37,** 424–435 (1995).
19. Karin, N. *et al. J. exp. Med.* **180,** 2227–2237 (1994).
20. Brocke, S. *et al. Nature* **365,** 642–644 (1993).
21. Platzer, C. *et al. Eur. J. Immun.* **22,** 1179–1184 (1992).

ACKNOWLEDGEMENTS. This work was supported in part by the NIH, the Phil Allen Trust, the Emil Aaltonen Foundation, the Wellcome Trust, and by Neurocrine Biosciences Inc. We thank L. Brocke for direct sequencing of PCR products, and T. Stahelin of Ciba Geigy for his comments.

Augmented humoral and anaphylactic responses in FcγRII-deficient mice

Toshiyuki Takai*, Masao Ono†, Masaki Hikida*, Hitoshi Ohmori* & Jeffrey V. Ravetch†

* Department of Biotechnology, Faculty of Engineering, Okayama University, Tsushima-Naka, Okayama 700, Japan
† Division of Molecular Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA

Despite its widespread distribution on both lymphoid and myeloid cells, the biological role of the low-affinity immunoglobulin-G receptor, FcγRII, is not fully understood. Defects in this receptor or its signalling pathway in B cells result in perturbations in immune-complex-mediated feedback inhibition of antibody production[1–4]. We now report that FcγRII-deficient animals display elevated immunoglobulin levels in response to both thymus-dependent and thymus-independent antigens. Additionally, the effector arm of the allergic response is perturbed in these mice. Mast cells from FcγRII$^{-/-}$ are highly sensitive to IgG-triggered degranulation, in contrast to their wild-type counterparts. FcγRII-deficient mice demonstrate an enhanced passive cutaneous anaphylaxis reaction, the result of a decreased threshold for mast-cell activation by FcγRIII cross-linking. These results demonstrate that FcγRII acts as a general negative regulator of immune-complex-triggered activation *in vivo* for both the afferent and efferent limbs of the immune response. Exploiting this property offers new therapeutic opportunities for the treatment of allergic and autoimmune disorders.

Immunoglobulin Fc receptors (FcRs) constitute a family of haematopoietic cell-surface molecules capable of eliciting intracellular signals and triggering numerous effector responses upon crosslinking by their ligand, the antibody–antigen complex[7]. Types I and III FcRs for IgG (FcγRI, FcγRIII) are expressed primarily on cells of the myeloid lineage and mediate effector functions, including phagocytosis, antibody-dependent cellular cytotoxicity and the release of inflammatory mediators, whereas type II receptors (FcγRII) are expressed on both myeloid and lymphoid lineages. *In vitro*, FcγRII inhibits the activation signal generated by the B-cell antigen receptor[1–4], mediated in part through the recruitment and activation of protein tyrosine phosphatases[5,6]. Similarly, reconstitution studies have suggested that FcγRII can inhibit activation through other, immune receptor tyrosine activation motif (ITAM)-containing receptors[8]. We therefore investigated the *in vivo* consequences of disrupting this inhibitory pathway.

Mice homozygous for FcγRII$^{-/-}$ were generated (Fig. 1*a*,*b*), which developed normally and were fertile. The absence of messenger RNA (not shown) and protein (Fig. 1*c*) for FcγRIIB was demonstrated by northern and western blotting, respectively; the absence of membrane bound FcγRIIB was demonstrated by flow cytometry of splenic B cells (Fig. 1*d*).

Expression of FcγRIIB on effector cells has been shown to depend on their state of activation[9,10]. Resident peritoneal macrophages (Fig. 1*e*) and bone-marrow-derived mast cells (Fig. 1*f*) reveal a >90% reduction in surface staining with the monoclonal antibody 2.4G2 in FcγRII$^{-/-}$ mice (Fig. 1*e*,*f*), whereas thioglycollate-elicited macrophages retain 80% of their 2.4G2 staining (data not shown).

Development of myeloid and lymphoid cell lineages were not affected by the loss of FcγRII, as revealed by flow cytometric analysis of cells derived from the spleen, bone marrow, thymus and peritoneal cavity using a panel of lineage-specific markers (data not shown).

Crosslinking of surface IgM on resting B cells *in vitro* induces a proliferative response only when anti-μ $F(ab')_2$ is used as the stimulating antibody; intact antibody fails to stimulate B cell receptor-mediated cellular activation[1]. In contrast, B cells derived from FcγRII$^{-/-}$ proliferate in response to both intact anti-μ and $F(ab')_2$ (Fig. 2*a*). The significance of this pathway *in vivo* was determined by immunizing wild-type and FcγRII$^{-/-}$ animals with sheep red blood cell (SRBC) or trinitrophenol keyhole limpet haemocyanin (TNP–KLH) as thymus-dependent antigens (Fig. 3*a*,*b*) or trinitrophenol lipopolysaccharide (THP–LPS) or TNP–Ficoll as thymus-independent antigens (Fig. 3*c*,*d*). In all cases, FcγRII$^{-/-}$ showed significantly higher antibody titres than those of wild-type littermates (Fig. 3). This enhancement was evident after 14 days and persisted during the secondary response. Anti-SRBC IgM and IgG plaque-forming cell responses of splenocytes derived from deficient mice were significantly higher than those of wild-type mice at four days after the second immunization (data not shown). Isotype-specific enzyme-linked immunosorbent assays (ELISAs) indicated that the immunoglobulin isotypes that contribute to these differences were IgM, IgG1, 2a, 2b, 3 and IgA (data not shown).

Recent *in vitro* reconstitution studies in mast cell lines has suggested that FcγRII could inhibit FcR-triggered activation[8]. Bone-marrow mast cells derived from FcγRII-deficient or wild-type mice were stimulated through FcεRI or FcγRIII (Fig. 2*b*). Degranulation and serotonin release in response to IgE crosslinking were similar in wild-type and FcγRII-deficient mice. In contrast, wild-type mast cells show little degranulation or serotonin release above background upon crosslinking of FcγRII/III, whereas mast cells from FcγRII-deficient mice showed a substantial activation upon the same stimulation (Fig. 2*b*). Hence, FcγRII is sufficient substantially to repress mast-cell activation triggered by FcγRIII, but has no direct effect on FcεRI-mediated activation. Loss of FcγRII thus renders these cells sensitive to IgG-mediated triggering. IgG1-mediated passive cutaneous anaphylaxis (PCA)